

In the Specification:

Please amend the specification as shown:

Please delete the paragraphs on page 19, lines 13-29 and replace them with the following paragraphs:

Figure 9a is a diagram showing the maps of the plasmids pTip-NH1, pTip-CH1, pTip-LNH1, pTip-LNH1, pTip-NH2, pTip-CH2, pTip-LNH2, and pTip-LCH2. The function of each region and the maps of the plasmids are shown; 6xHis tags disclosed as SEQ ID NO: 168.

Figure 9b shows the DNA sequence of the pTip-NH1 or the pTip-LNH1 from a *TipA* gene promoter sequence or a *TipA-LG10* promoter sequence via a multiple-cloning site to a *ThcA* gene transcription termination sequence; Figure discloses SEQ ID NO: 108 coding SEQ ID NO: 109; short DNA sequence is SEQ ID NO: 110.

Figure 9c shows the DNA sequence of the pTip-CH1 or the pTip-LCH1 from a *TipA* gene promoter sequence or a *TipA-LG10* promoter sequence via a multiple-cloning site to a *ThcA* gene transcription termination sequence; Figure discloses SEQ ID NO: 111 coding SEQ ID NO: 112; short DNA sequence is SEQ ID NO: 113.

Figure 9d shows the DNA sequence of the pTip-NH2 or the pTip-LNH2 from a *TipA* gene promoter sequence or a *TipA-LG10* promoter sequence via a multiple-cloning site to a *ThcA* gene transcription termination sequence; Figure discloses SEQ ID NO: 114 coding SEQ ID NO: 115; short DNA sequence is SEQ ID NO: 116.

Figure 9e shows the DNA sequence of the pTip-CH2 or the pTip-LCH2 from a *TipA* gene promoter sequence or a *TipA-LG10* promoter sequence via a multiple-cloning site to a *ThcA* gene transcription termination sequence; Figure discloses SEQ ID NO: 117 coding SEQ ID NO: 118; short DNA sequence is SEQ ID NO: 119.

Figure 10 is a diagram showing the maps of pTip-CH1.1 (SEQ ID NO: 120), pTip-LCH1.1 (SEQ ID NO: 121), pTip-CH2.1 (SEQ ID NO: 122), and pTip-LCH2.1 (SEQ ID NO: 123);

Please delete the paragraphs on page 20, lines 7-9 and replace it with the following paragraphs:

Figure 12 is a diagram showing a *TipA* gene promoter sequence (SEQ ID NO: 107);

Figure 13 is a diagram showing the modification of a *TipA* gene promoter (SEQ ID NO: 124) to a *TipA*-*LG10* promoter (SEQ ID NO: 125);

Please delete the paragraphs on page 20, lines 13-21 and replace it with the following paragraphs:

Figure 15 is diagram showing the amino acid sequences of five motifs (Motif IV, Motif I, Motif II, Motif III, and C-terminal motif) that are conserved in Rep proteins among pRE8424 (SEQ ID NOS: 126-130), pAP1 (SEQ ID NOS: 131-135), pBL1 (SEQ ID NOS: 136-139), pJV1 (SEQ ID NOS: 140-144), pIJ101 (SEQ ID NO: 145-149), and pSN22 (SEQ ID NO: 150-154). A tyrosine residue allegedly important for the function of the Rep protein is boxed;

Figure 16 is a diagram showing an especially conserved DNA sequence, of sequences likely to be the DSOs of the pRE8424 (SEQ ID NO: 155), the pAP1 (SEQ ID NO: 156), the pBL1 (SEQ ID NO: 157), the pJV1 (SEQ ID NO: 158), the pIJ101 (SEQ ID NO: 159), and the pSN22 (SEQ ID NO: 160);

Figure 17 is a diagram showing the SSO of the pRE8424 (SEQ ID NO: 161), that is, a sequence of nucleotide Nos. 5268 to 5538 in SEQ ID NO: 90 in the sequence listing, and a possible secondary structure;

Please delete the paragraph on page 20, line 24-27 and replace it with the following paragraph:

Figure 19 is a diagram showing the DNA sequence of *TipA*-*LG10p*-MCS-*ALDHt* or *Nit*-*LG10*-MCS-*ALDHt*. A wild-type -10 region sequence of a *TipA* gene promoter is CAGCGT, and a -10 region sequence of a *Nit* promoter is TATAAT. These sequences are boxed, respectively; Figure discloses SEQ ID NOS: 162-167, respectively, in order of appearance.

Please delete the paragraph on page 21, lines 4-8 and replace it with the following paragraph:

Figure 21 is a photograph showing a result obtained by the following procedures: *PIP* and *GFP* genes are incorporated into two vectors that do not cause incompatibility with each other, PIP and GFP expressed in a single *R. erythropolis* strain JCM3201 cell are purified and analyzed by SDS polyacrylamide electrophoresis, followed by the staining of the gels with Coomassie Brilliant Green G-250. 6xHis tags disclosed as SEQ ID NO: 168.

Please delete the paragraph on page 29, lines 15-27 and replace it with the following paragraph:

Next, primers represented by SEQ ID NOs: 23 and 24 in the sequence listing were used to perform amplification by PCR with a plasmid pRSET-PIP (Tamura et al., FEBS Lett. 398 101-105 [1996]; hereinafter, abbreviated to PIP) as a template. The primer represented by SEQ ID NO: 24 in the sequence listing is designed so that 6xHis tag (SEQ ID NO: 168) is attached to the C terminus of a PIP protein in order to eliminate the termination codon of the *PIP* gene and facilitate protein purification. The 6xHis tag (SEQ ID NO: 168) is a consecutive sequence consisting of six consecutive histidine residues, and a protein fused with this tag exhibits high affinity for a nickel ion or the like. Thus, the protein is readily purified by metal chelate chromatography that employs the nickel ion or the like (Crowe et al., Methods Mol. Biol. 31 371-387 [1994]). This 0.9-kb DNA fragment containing the *PIP* gene was doubly digested with restriction enzymes *Nco*I and *Spe*I and subcloned into the *Nco*I and *Spe*I sites of the pHN150u. Consequently, a plasmid containing the ORF of the *PIP* gene located immediately downstream of the *TipA* gene promoter sequence was constructed and designated as pHN151u.

Please delete the paragraph on page 45, lines 9-20 and replace it with the following paragraph:

Although both of the pHN171 and the pHN348 were expression vectors where a *PIP* gene, a reporter gene, was introduced into the MCS of the pTip vector (see Reference Example), the difference between them is only in a transformation marker: a tetracycline resistance gene for the pHN171 and a chloramphenicol resistance gene for the pHN348. In any of the plasmids, a ribosome-binding site sequence originally located downstream of the *TipA* gene promoter (*TipA*-RBS) is altered into a bacteriophage *gene 10*-derived ribosome-binding site sequence having good translation efficiency (*TipA-LG10* promoter; see Reference Example). A 6xHis tag (SEQ ID NO: 168) is adapted to be attached to the C terminus of PIP in order to facilitate protein purification. The 6xHis tag (SEQ ID NO: 168) is a consecutive sequence consisting of six consecutive histidine residues, and a protein fused with this tag exhibits high affinity for a nickel ion or the like. Thus, the protein is readily purified by metal chelate chromatography that employs the nickel ion or the like (Crowe et al., Methods Mol. Biol. 31 371-387 [1994]).

Please delete the paragraphs on page 54, line 3 to page 55, line 16 and replace them with the following paragraphs:

At first, primers represented by SEQ ID NO: 86 (sHN337) and 87 (sHN338) in the sequence listing were used to perform DNA amplification by PCR with the pHN187 (see Reference Example 1) as a template. The obtained 0.2-kb fragment contains the 5' end portion of the *GFP* gene. This fragment was digested with *Nco*I, and its 5' ends were phosphorylated. On the other hand, primers represented by SEQ ID NO: 88

(sHN339) and 89 (sHN340) in the sequence listing were used to perform DNA amplification by PCR with the pHN187 as a template. The obtained 0.5-kb fragment contains the 3' end portion of the *GFP* gene. This fragment was digested with *Bgl*I, and its 5' ends were phosphorylated. These two DNA fragments were simultaneously introduced into the *Nco*I/*Bgl*I sites of the pNit-QT1 and the pNit-RT1, respectively. The resulting plasmids were designated as pHN425 and pHN426, respectively. The pHN425 and the pHN426 contain the full-length *GFP* gene and are fused with a sequence for attaching a 6xHis tag (SEQ ID NO: 168) to the C terminus of GFP. Although the *Nco*I site present within the *GFP* gene is eliminated during the above-described procedures, the function of the GFP is not changed.

*R. erythropolis* JCM3201 was cotransformed with the pHN425 and the pHN389, and a cotransformant was selected on a medium containing both tetracycline and chloramphenicol. Alternatively, *R. erythropolis* JCM3201 was cotransformed with the pHN426 and the pHN409, a cotransformant was selected on a medium containing both tetracycline and chloramphenicol. As a control experiment, *R. erythropolis* JCM3201 was separately transformed with the pHN425, the pHN426, the pHN389, and the pHN409. These six types of transformants were allowed to express PIP and GFP as described in Example 1, which were in turn purified by metal chelate chromatography that employs a nickel ion. SDS polyacrylamide electrophoresis for the purification of the recombinant proteins and for the samples before and after purification was conducted by the following procedures. The 6xHis tag (SEQ ID NO: 168) was attached to the C terminus of the PIP, and purification was performed using the Ni-NTA Superflow (manufactured by QIAGEN) according to the instruction.

Hereinafter, a specific purification method will be illustrated. Procedures for the purification were performed at 4°C. The bacterial cells (in 20 ml culture solution) where the protein was expressed were recovered and suspended in 1 ml of the NT-Buffer (50 mM Tris-HCl (pH 8.0), 100 mM sodium chloride, and 1 mM dithiothreitol), to which 1 g of glass beads (with a diameter of 0.105 to 0.125 mm) was then added. These beads were put into a reciprocating shaking motion at a speed of 6 m/sec for 20 seconds in the Fast-prep FP120 (manufactured by SAVANT) to destroy the cells. Following centrifugation at 20,000xg, 700 µl of the resulting supernatant was supplemented with the Ni-NTA Superflow equilibrated in advance with the NT-Buffer to bring the bed volume to 40 µl. While this was stirred by rotation for 1 hour, the Ni-NTA Super flow beads were bound to the protein attached to the 6xHis tag (SEQ ID NO: 168). These beads were washed four times with the NT-Buffer and then suspended three times in 120 µl of the NTE-Buffer (50 mM Tris-HCl (pH 7.0), 100 mM sodium chloride, 1 mM dithiothreitol, and 400 mM imidazole), thereby eluting the protein attached to the 6xHis tag (SEQ ID NO: 168) from the beads. A 10-µl aliquot of the sample was subjected to 12% SDS

polyacrylamide electrophoresis according to an ordinary method. A result of staining the gel with Coomassie Brilliant Green G-250 after analysis by SDS polyacrylamide electrophoresis is shown in Figure 21.

Please delete the Table 1 header on page 57 and replace it with the following header:

Table 1

Table 1 Main plasmids used in the present invention

(6xHis tags disclosed as SEQ ID NO: 168)